REMARKS

The present response is intended to be fully responsive to all points of rejection raised by the Examiner and is believed to place the application in condition for allowance.

Applicants assert that the present invention is new, non-obvious and useful. Prompt consideration and allowance of the claims is respectfully requested.

Applicants submit that references to the subject Application are based on U.S. Patent Application Publication No.: US 2002/0102728 A1.

Status of Claims

Claims 24-29 are pending. Claims 24-29 have been rejected.

Claim 29 has been canceled without prejudice or disclaimer. In making this cancellation without prejudice, Applicants reserve all rights in these claims to file divisional and/or continuation patent applications.

CLAIM REJECTIONS

35 U.S.C. § 112 Rejections

In the Office Action, the Examiner maintained the rejection of claims 24-29 under 35 U.S.C. § 112, first paragraph, as failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants disagree.

i. The Examiner alleged the collagen sponge used in the Examples is osteoinductive, citing pages 158-159 of Wolfe *et al.*, Med. Prog. Technol., 1994, 20:155-168 in support. Applicants disagree.

Wolfe *et al.* define "Osteoinduction" as "the formation of new bone by the active recruitment of host pluripotent cells that differentiate into chondroblasts and osteoblasts" (Wolfe *et al.*, page 155, left-hand column starting 3 lines from the bottom to first line of right-hand column). The osteoinductive demineralized bone matrix (DBM) implants described in Wolfe *et al.* on pages 158-159 are not collagen sponge carriers of the subject Invention. Wolfe *et al.* describe collagen as a delivery vehicle "*incapable of inducing osteogenesis in vivo*" (emphasis added; Wolfe *et al.* page 160, left-hand column lines 8-9

from the bottom of the page). Thus, contrasting the Examiner's assertion, Wolfe *et al.* discloses that a collagen sponge is not osteoinductive, as it does not form new bone by the active recruitment of host pluripotent cells.

Moreover, Examples 3, 8, 9, 11, 14 and 15 corroborate the disclosure of Wolfe *et al.* that a collagen sponge is not osteoinductive. Experimental controls implanting collagen sponge alone or collagen sponge loaded with cells/virus not expressing BMP-2 reveal a lack of cell differentiation or bone formation in the absence of BMP-2. The skilled artisan would therefore recognize that the collagen sponge carrier of the subject Application is not osteoinductive.

The Examiner appears to be alleging that any implant becomes osteoinductive when it contains osteoinductive factors, and therefore a collagen sponge is osteoinductive. Applicants disagree.

Applicants state that subject claims relate to methods of inducing bone formation at a site of bone infirmity in a human comprising (a) transforming cells with DNA encoding an osteogenic protein (BMP-2); (b) culturing these cells under conditions that enable expression of BMP-2; and (c) implanting these cells at the site of bone infirmity in the absence of exogenously supplied osteoinductive matrix.

Applicants assert that the inherent properties of the collagen sponge as an inert delivery vehicle, as described *supra*, are not modified by mounting cells expressing an osteoinductive protein onto the sponge. In support, Wolfe *et al.* points out that it is the osteogenic proteins that are responsible for the osteoinductive capacity of demineralized bone matrix implants; Wolfe *et al.* further describes other non-osteoinductive carrier substances to include *collagen*. In particular, Wolfe *et al.* states "solid *collagens in a sponge-like form* were used for the first time *to function as a delivery system* for an osteoinductive substance". (*See*, Wolfe *et al.* Abstract; pages 158-160; page 160 last line in left-hand column to lines 1-2 of right-hand column) Thus, the collagen sponge itself is not and does not become osteoinductive but acts as a delivery vehicle for cells expressing an osteoinductive protein.

Use of a non-osteoinductive matrix such as a collagen sponge, is described in the subject Application as being "for supporting the composition and providing a surface for bone, cartilage, muscle, nerve, epidermis and/or other connective tissue growth....The matrix

may provide slow release of the expressed protein and differentiated cells and/or the appropriate environment for presentation thereof." (paragraph 17) Further, "The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties." (see subject Application, paragraph 18) Applicants emphasis that implantation of *mesenchymal stem cells (MSC) expressing osteogenic growth factor BMP-2* with a *non-osteoinductive matrix*, such as a collagen sponge, provides a mechanism of delivery of cells possessing paracrine and autocrine properties and which express an osteogenic BMP-2 protein to a site of bone infirmity. Osteoinductive properties/activities observed in segmental defects, for example, are *provided by the osteogenic proteins and the response to these proteins by MSC* and surrounding environment/tissue.

Further, contrary to the Examiner's allegation, the use of a collagen sponge as a delivery vehicle, does not entail pre-loading BMP-2 into the collagen sponge prior to mounting cells on the sponge (see Examples 3, 8, 9, 11, 14 and 15).

Thus, Applicants maintain the assertion that a collagen sponge carrier is not osteoinductive and therefore, Examples support claimed subject matter of, *inter alia*, claim 24 (c) "implanting said cultured mesenchymal stem cell in the absences of an exogenously supplied osteoinductive matrix"

The Examiner alleged the reference Moutsatsos *et al.* Mol. Therapy. 2001 3(4):449-461, discloses that only co-implantation with an osteoinductive matrix leads to regeneration of functional bone, since there is no teaching in the reference of implanting cells without a matrix. Applicants disagree.

Moutsatsos *et al.* describes that regulated expression of "*osteogenic growth factor BMP-2*" controlled both bone formation and bone regeneration. (*See*, Moutsatsos *et al.* Abstract) Moutsatsos *et al.* states that collagen sponges "were used to deliver the cells into the transplantation site" (*See*, Moutsatsos *et al.* page 451, left-hand column lines 32-33) *Formation of bone tissue at these sites was dependent on expression of BMP-2 protein*, as inhibition of BMP-2 expression in the presence of DOX resulted in lack of bone formation. Further, contrary to the Examiner's assertion that "there is no teaching in the reference of implanting cells without a matrix", Moutsatsos *et al.* describes that "*It is noteworthy that*

injection of C9 cells [MSC cells expressing BMP-2] directly under the skin *had also formed ossicle similar to collagen-delivered C9 transplants*." (emphasis added; *See*, Moutsatsos *et al.* page 45, left-hand column lines 28-31; Figs. 4e-4j)

Hence, Moutsatsos *et al.* like Wolfe *et al.* describe a collagen sponge as a matrix to function as a delivery system for an osteoinductive substance. The disclosures of Mousatsos *et al.* and Wolfe *et al.*, expressing knowledge in the art, support the claims of the subject Application wherein a method of bone formation comprises implanting MSC expressing BMP-2 protein in the absence of an exogenously supplied osteoinductive matrix, e.g. a collagen sponge, at a site of bone infirmity. For at least these reasons, one skilled in the art, based on the Specification as filed, the guidance provided in the Example, and knowledge in the art will recognize a collagen sponge as a non-osteoinductive matrix. Thus, claims 24-28 are proper under 35 USC 112, first paragraph.

ii. The Examiner alleged that the subject Application fails to provide sufficient guidance for a skilled artisan on how to perform the claimed methods alleging "bone formation cannot occur by simply implanting MSCs in the absences of a support matrix". Applicants disagree.

The subject Application describes the DNA coding for osteoinductive proteins and other useful proteins (paragraphs 9, 10 and 11), cells for transformation with DNA (paragraph 13), vectors for expression of DNA (paragraph 14), uses of the instant invention for regeneration of bone (paragraph 16), and use of a combination of cells and a matrix (paragraphs 17 and 18), wherein a matrix is provided as support surface for bone growth. Examples 1, 2 and 13 describe implantation of MSC expressing BMP-2 in the absence of a matrix results in bone induction. Examples 3, 8, 9, 11, 14 and 15 describe implantation of MSC expressing BMP-2 mounted on collagen sponges/gels results in formation of new bone tissue including gap-healing of a radial segmental defect. Thus, the subject Application describes and exemplifies bone formation as a result of implantation of MSC expressing BMP-2 in the absence of a matrix and in the absence of an exogenously supplied osteoinductive matrix.

The Examiner alleged that the art teaches bone growth can be "accomplished only by using a support matrix" (Bruder *et al.* and Leach *et al.*). Applicants disagree.

Bruder *et al.* presents hypotheses and rules that "that appear to govern all processes involving MSC-medicated bone formation" (see Bruder *et al.* Summary page 292). In stark contrast, the subject Application *exemplifies* bone induction in the absences of a matrix (Examples 1, 2 and 13). Moreover, as argued *supra*, the disclosure of Moutsatsos *et al.* Mol. Therapy. 2001, supports this result.

Applicants assert that while Leach *et al.* may suggest association of cells with carriers to be effective in bone formation, Leach *et al.* no where states that growth can be accomplished *only* by using a support matrix, as stipulated by the Examiner. In fact, Leach *et al.* states that "Systemic infusion or injection of inductive factors can be successful" in bone regeneration (see Leach *et al.* page 1016, right-hand column, lines 2-3).

For at least these reasons, one skilled in the art, based on the Specification as filed, the guidance provided in the Example, and knowledge in the art will recognize the potential for bone formation by implanting MSCs in the absence of a matrix. Thus, claims 24-28 are proper under 35 USC 112, first paragraph.

iii. The Examiner alleged that the instant Specification and Examples fail to provide sufficient guidance for a skilled artisan on how to perform the claimed methods in that the Specification provides only two examples (*See* Office Action page 4). Applicants disagree.

Applicants direct the Examiner's attention to Examples 1-15 described in the subject Application, as briefly described below:

Example 1 (page 3) describes the ability of MSC expressing BMP-2 to develop into newly formed ectopic bone in the absence of a matrix.

Example 2 (pages 3 and 4) describes *in vitro* and *in vivo* regulated expression of BMP-2 in MSC demonstrating in vitro BMP-2 expression, in vivo survival of MSC expressing BMP-2 in 3 mm segmental defects.

Example 3 (page 4) describes MSC expressing BMP-2 and mounted on collagen sponges (non-osteoinductive), further implanted at sites of segmental defects (2.5 mm, 3 mm, 3.5 mm) and *observed for up to 6 weeks*. Results show newly-formed bone (increased radiopacity) compared with a lack of healing in control groups (collagen only and segmental defect only). New bone was comprised of bone trabeculas and fatty bone marrow.

Example 4 (page 4) describes successful viral gene delivery into osteoprogenitor cells.

Example 5 (page 4) describes comparison of bone formation when implanting cells expressing BMP-2 versus those expressing BMP-2 and parathyroid hormone receptor (PTHR) (autocrine effect). Bone formation was observed with implants expressing BMP-2 but not those expressing BMP-2 and PTHR.

Example 6 (pages 4 and 5) describes systemic administration of recombinant BMP-2 in adult mice results in increased physical potency and excluded adverse systemic effects of BMP-2 on the CNS.

Example 7 (page 5) describes MCS as suitable hosts for *in vitro* transfection by adenoviral vectors expressing BMP-2.

Example 8 (pages 5 and 6) describes cells expressing BMP-2 and mounted on a collagen sponge (non-osteoinductive) were implanted into a *2.5 mm radial segmental defect* and *observed for 6-8 weeks*. Greater gap healing was observed with MSC cells expressing BMP-2 than with CHO cells expressing BMP-2 (CHO lack the ability to differentiate into osteoblasts.).

Example 9 (pages 6 and 7) describes cells expressing BMP-2 and studied in vitro (BMP-2 expression) and in vivo (mounted on collagen sponges-non-osteoinductive), *implanted into 2.5 mm segmental defects* and *observed for 6-8 weeks*). Results demonstrated BMP-2 in vitro expression and highest values of gap repair and organized bone formation with MSC expressing BMP-2 as compared to collagen sponges alone.

Example 10 (pages 7 and 8) describes osteocalcin synthesis stimulated by BMP-2 in bone marrow stromal cells.

Example 11 (pages 8-13) describes genetic engineering of MSC expressing BMP-2 and enhanced bone repair (organized and oriented bone formation) of three different MSC clones, mounted onto collagen sponges (non-osteoinductive) and implanted into *2.5 mm segmental defects*. Observations were carried out over *8 weeks*.

Example 12 (pages 13 and 14) describes extra skeletal effects of BMP-2 administered systemically.

Example 13 (pages 14 and 15) describes the use of encapsulated BMP-2 expressing cells injected into a subcutaneous area in the back and the resultant bone and cartilage formation observed.

Examples 14 (pages 15-18) describes MSC expressing BMP-2 from an adenovirus and further, transplanting such cells as part of a collagen gel (non-osteoinductive matrix) into a subcutaneous area demonstrated that *bone formation was dependent on BMP-2 expression* (*observed after 10 and 20 days*).

Example 15 (pages 18- 19) describes intramuscular transplantation of MSC expressing BMP-2, mounted on collagen sponges (non-osteoinductive) to study effects of paracrine and autocrine responses after 10 and 20 days . Results disclose cell surface receptors affects differentiation pathway of pluoripotent cells.

As listed above, and as described in greater detail in the subject Application as filed, the subject Application clearly provides guidance for a skilled artisan on how to perform the claimed methods. Further, Examples demonstrate successful induction of organized bone formation at sites of bone infirmity upon implanting MSC expressing BMP-2 in the absence of an exogenously supplied osteoinductive matrix at a site of bone infirmity.

Based on the arguments presented *supra*, Applicants submit that one skilled in the art, based on the Specification as filed, the guidance provided in the Example, and knowledge in the art will know how to make and use the claimed compositions. Applicants submit that Examples cannot serve to limit the scope of the claimed invention, but serve rather to provide guidance for how to make and use the scope of the claimed invention.

Accordingly, Applicants respectfully assert that claims 24-28 are proper under 35 USC 112 and request that the rejections be withdrawn.

Conclusion

In view of the foregoing amendments and remarks, Applicants assert that the pending claims are allowable. Their favorable reconsideration and allowance is respectfully requested.

Should the Examiner have any question or comment as to the form, content or entry of this Amendment, the Examiner is requested to contact the undersigned at the telephone number below. Similarly, if there are any further issues yet to be resolved to advance the prosecution of this application to issue, the Examiner is requested to telephone the undersigned counsel.

Please charge any fees associated with this paper to deposit account No. 50-3355.

Respectfully submitted,

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